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14. ABSTRACT

Genetic factors influence risk of exposure to trauma suggesting a role for personality traits like neuroticism, impulsivity, and/or preexisting conditions such as anxiety or depression in developing PTSD. Calcyon is an excellent candidate gene for investigating a potential relationship between impulsivity and PTSD. We established a repository of cell lines from ~300 PTSD-positive and control military personnel and veterans with mild to severe combat exposure. Each participant was extensively interviewed and tested with respect to trauma exposure, social support, medical history, personality traits and symptoms by clinicians specializing in PTSD, and will be genotyped for ancestry. We are on target for determining genotypes at 8 loci in the calcyon gene which is strongly associated with attention deficit hyperactivity disorder (ADHD) and determine haplotypes using the program PHASE within the next four months. Single locus and haplotype analyses of CAPS sores will be based on linear regression models and will include combat exposure, ethnicity, impulsivity and social environment as covariates. In addition, gene x environment interactions will directly be tested. All of our findings on the effect of polymorphisms in calcyon to PTSD susceptibility in the combat exposed military personnel will be submitted for publication, and if positive could be useful in prognostic screen of combat-worthy soldiers.

15. SUBJECT TERMS
calcyon; haplotype, association study; combat exposure; ethnicity; environment; covariates

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INTRODUCTION:

Post-traumatic stress disorder (PTSD) develops in a subset of patients exposed to traumatic stress. Twin studies with Vietnam Veterans revealed a role for genetic predisposition in trauma associated anxiety, re-experiencing trauma and avoidance of stimuli related to trauma (True et al., 1993; Goldberg et al., 1990). Genetic factors also influence risk of exposure to trauma suggesting a role for personality traits like neuroticism, impulsivity, and/or preexisting conditions such as anxiety or depression in developing PTSD (Lyons et al., 1993; Stein et al., 2002; Jang et al., 2003).
To date, evidence from family and candidate gene association studies has been accumulating for a role of genes related to the hypothalamic-pituary-adrenal axis, the limbic amygdalar frontal pathway mediating fear processing, and the ascending brainstem locus coeruleus noradrenergic system in PTSD (see recent review by (Norrholm and Ressler, 2009)). However, the genetic polymorphisms associated with risk and resilience for PTSD identified so far do not support a strong basis for a genetic main effect in PTSD and do not fully account for the genetic heritability of PTSD found in twin studies. Increasing evidence is accumulating for a strong environmental effect, and indicate that gene x environment interactions (such as e.g., data on social support or childhood abuse) should be included in genetic association studies. These studies therefore require a very carefully phenotyped study population.

A combination of data from human and murine studies supports the use of the calcyon as an excellent candidate gene for investigating a potential relationship between impulsivity and PTSD (Trantham-Davidson et al., 2008; Laurin et al., 2005; Heijtz et al., 2007; Loos et al., 2009; Dasbanerjee et al., 2008) (CB, unpublished data). The goal of the present study is to determine whether a DNA haplotype designated ‘C1’ in the *calcyon* locus strongly associated with attention deficit hyperactivity disorder (ADHD) could be useful as an unbiased screen for PTSD-prone soldiers. Subjects participating in the current study include active military personnel and veterans with mild to severe combat exposure. Each participant is extensively phenotyped with respect to trauma exposure, social support, medical history, personality traits and symptoms by clinicians specializing in PTSD, and will be genotyped for ancestry. This information will permit assessment of important covariates such as environmental factors, personality type, ethnicity, and premorbid disorders. The availability of unambiguous and unbiased prognostic information on susceptibility could prove invaluable in the assignment of soldiers to combat duty, possibly saving our country millions of dollars, and sparing the afflicted soldiers and their families, untold anguish. Further, if the hypothesis is validated by the study proposed here on *calcyon*, this simple genetic test could also be useful in the diagnosis and treatment of PTSD.

BODY:

- **Subjects**:

The current study includes 276 subjects who are active military personnel and veterans with mild to severe combat exposure. A key feature of our study design is that the subjects have been extensively interviewed and evaluated by Dr. Dewleen Baker, M.D., a specialist in PTSD. This process yielded valuable demographic information on the

subjects as well data on ethnic background, medical history and symptoms. Each subject completed the questionnaires Fagerstorm test for nicotine dependence, DAST, AUDIT, SF-36 Health survey, Combat exposure Scale, Traumatic events survey-R, International Physical Activity questionnaire, Beck Depression Inventory (BDI-II), Anger Scale - Retrospective Overt Aggression Scale, Cook-Medley Scale, STAXI-2, PDEQ-M, PDI-M, PPCQ-S, PSDQ, PLC-C, Cloninger personality Scale, and the CTQ. In addition, CAPS (clinician-administered PTSD scale), SCID, and HamD tests were conducted on all participants. All data was entered into a web-based databank and extensive data entry QC has been performed.

All subjects also consented to genetic testing and provided a blood sample for DNA analyses. Genomic DNA was isolated from lymphocytes from each subject and successfully transformed into lymphoblastoid cell lines to provide an unlimited source of DNA. All subjects also provided a urine sample for a toxicological screen.

- **Preliminary Data**

Analysis of the CAPS resulted in the following diagnoses:

86 with no PTSD (CAPS <=30)
55 with non discordant phenotype (ndp) (CAPS 31-64)
135 with PTSD (CAPS >=65)

PTSD scores in this group of subjects significantly correlated with combat exposure (Spearman's rho =0.4, N=233, p<0.001). However, there was no correlation with gender as 93% of the subjects are male, and the gender distribution is the same between the 3 diagnostic groups (Table1).

	Gender	
DX	**male (%)**	**female (%)**
no PTSD	96	4
ndp	92	8
PTSD	91	9
Total	93	7

Self-identified ethnicity and race was available only from 57% and 70%, respectively. Interestingly, these data showed that the ancestral background of the subjects was not evenly distributed between the 3 diagnostic classes (Tables 2 and 3).

	Ethnicity (N)			
DX	Hispanic	Non-Hispanic	NA	Total
no PTSD	26	22	38	86
ndp	13	14	28	55
PTSD	49	32	54	135
Total	88	68	120	276

DX	Race (N)							
	European American	African American	Asian	Native Americans	Hawaiian/ Pacific Islander	Other	NA	Total
no PTSD	47	2	6		2	3	26	86
ndp	32	2	3	2			16	55
PTSD	60	14	9	2	2	7	41	135
Total	139	18	18	4	4	10	83	276

It is well known that population stratification, if not accounted for, will lead to false positive (and negative) findings in genetic association studies. Therefore, to strengthen our study, we will genetically determine the ancestry of the study subjects using a small panel of ancestry-informative markers (AIMs) and prior to genotyping of the calcyon C1 haplotype. Publicly available genotype data including over 0.5M markers (Illumina 650y array) of 944 subjects of the Human Genome Diversity Panel (Li et al., 2008) was used as a reference population to select a set of 41 highly informative markers that were able to distinguish the 7 major world regions/continents (Nievergelt et. al, in prep.). These markers were selected to be suitable for multiplexing on the ABI SNPlex genotyping system (https://products.appliedbiosystems.com) and genotyping is currently ongoing.

We are currently designing a second ABI SNPlex genotyping pool with 45 SNPs covering five PTSD candidate genes, including 8 SNPs in calcyon.

KEY RESEARCH ACCOMPLISHMENTS

- Carefully phenotyped subjects for gene association and gene X environment interaction studies on susceptibility to PTSD related to combat exposure.
- Established cell lines from each participant for an unlimited source of subject DNA for future studies.

REPORTABLE OUTCOMES:

Repository of cell lines from ~300 extensively phenotyped PTSD-positive and control combat exposed subjects.

CONCLUSION:

We are on target to determine genotypes at 8 loci in the C1 haplotype of the calcyon gene in ~300 subjects and determine haplotypes using the program PHASE within the next four months. Single locus and haplotype analyses of CAPS sores will be based on linear regression models and will include combat exposure, ethnicity, impulsivity and social environment as covariates. In addition, gene x environment interactions will directly be tested. All of our findings on the effect of polymorphisms in calcyon to PTSD susceptibility in combat exposed military personnel will be submitted for publication. If positive could be useful in prognostic screen of combat-worthy soldiers.

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